

Salinity and Alkaline pH in Irrigation Water Affect Marigold Plants: II. Mineral Ion Relations

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Abstract. Scarcity of good-quality water for landscape irrigation is a major concern in arid and semiarid regions as a result of the competition with the urban population. Competing claims from urban, agricultural, environmental, and industrial groups leaves less water or water of lower quality for use in landscape maintenance. Although degraded waters, high in both salinity and alkaline pH, may challenge plant establishment and growth, these waters must be considered as valuable alternatives to the use of fresh water resources for landscape sites. The objective of the present study was to determine the effect of irrigation with saline water, with and without pH control, on the mineral ion relations of three marigold cultivars: Flagstaff, Yellow Climax, and French Vanilla. Treatments were five electrical conductivities of irrigation water (EC_w): 2, 4, 6, 8, and 10 $dS\cdot m^{-1}$, and two pH levels: 6.4 and 7.8. Plants of 'French Vanilla' and flowering stems of 'Flagstaff' and 'Yellow Climax' were harvested at flower maturity. Leaves of the taller cultivars, Flagstaff and Yellow Climax, were collected separately from the main axis and from the lateral stems, whereas in 'French Vanilla', leaves were combined. Total sulfur, total phosphorus, Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Cl^- , Fe^{2+} , Zn^{2+} , Cu^{2+} , and Mn^{2+} concentrations in leaf and stem tissues were determined. The three marigold cultivars were strong Ca^{2+} -accumulators and this response was more evident at the lower pH level. However, leaf Ca^{2+} tended to decrease as salinity increased despite a threefold increase in substrate Ca^{2+} . Leaf Mg^{2+} increased as salinity increased and main stem leaves of the taller cultivars accumulated more Mg^{2+} than leaves on the lateral branches. The reverse was true for leaf K^+ ; leaves on the lateral branches were stronger K^+ -accumulators than those on the main stem. Potassium concentrations in leaves of marigold irrigated with waters at pH 6.4 tended to decrease as EC_w increased. Marigold seems to possess an efficient Na^+ exclusion mechanism, which restricts Na^+ accumulation in the leaves. Patterns of total phosphorus accumulation in leaf tissues were not consistent over the range of EC_w treatments. Among the micronutrients, Fe^{2+} and Mn^{2+} tended to be partitioned to the younger rather than the older leaves. The decrease in marigold growth was associated with nutrient ion imbalance as demonstrated by the reduction in K^+ concentration and the increase in Mg^{2+} and Cl^- in leaf tissue. Despite the reduction in growth, the aesthetic value of the cultivars was not detrimentally affected by application of saline waters with EC_w values as high as 8 $dS\cdot m^{-1}$.

Water availability and quality are issues of major concern in reference to irrigation of urban landscapes resulting from the competition with a rapidly growing population that requires vast volumes of good-quality water (Kjelgren et al., 2000). Landscape irrigation accounts for 10% of total volume in the summer rainfall region of the United States,

whereas in the arid western regions, irrigation of landscape accounts for nearly 50% (Kjelgren et al., 2000). One strategy for conservation of fresh water resources is the use of low-quality water for landscape irrigation. These degraded water resources may be saline and/or alkaline and also may contain specific ions that are toxic for plant growth (Reboll et al., 2000). Salinity, caused by Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , and SO_4^{2-} , together with alkalinity, resulting from HCO_3^- and CO_3^{2-} , have direct toxic effects on plant physiology and/or affect plant growth through osmotic effects and loss of nutrient availability.

Marigold, one of the most important ornamental plants, is valued in landscape settings and also as cut flowers (Nau, 1997). In a companion paper (Valdez-Aguilar et al., 2009b), we described the effect of increasing irrigation water salinity and pH on growth and aesthetic value of three cultivars of marigold,

'French Vanilla' (*Tagetes patula*) and 'Flagstaff' and 'Yellow Climax' (both *T. erecta*). The results showed that marigold is sensitive to electrical conductivity of irrigation water (EC_w) higher than 4 $dS\cdot m^{-1}$ and that growth reduction was greater when pH of irrigation water was 7.8 compared with 6.4. However, despite the growth reduction resulting from increasing salinity, plants were still of acceptable quality for landscape use even when EC_w was as high as 8 $dS\cdot m^{-1}$. High irrigation water pH, however, caused a significant loss of aesthetic value. The objective of the present study was to investigate the relationship between the growth reduction detected in marigold as an effect of irrigation with saline water and high pH and ion accumulation to understand potential ion imbalances.

Materials and Methods

The experiment was conducted in a greenhouse at the U.S. Salinity Laboratory in Riverside, CA. Growth conditions and additional experimental details are provided in the companion paper (Valdez-Aguilar et al., 2009b). Three marigold cultivars, French Vanilla, Yellow Climax, and Flagstaff, were used for the present study. Treatments consisted of five EC_w simulating the ionic composition of Colorado River water (Table 1) and were based on predictions of what long-term compositions would be on further concentration resulting from plant water extraction and evaporation (Suarez and Simunek, 1997). The EC_w treatments were 2, 4, 6, 8, and 10 $dS\cdot m^{-1}$ and two pH levels: 6.4 (± 0.1) and 7.8 (± 0.2). Irrigation water was acidified by adding HNO_3 until the target nitrogen concentration was reached and thereafter with H_2SO_4 . Nutrient concentration of the irrigation solutions was maintained constant and was prepared as described in a companion paper (Valdez-Aguilar et al., 2009b). The higher pH was the normal pH of Riverside tap water. At harvest, average alkalinity was 0.47 ± 0.05 and 1.82 ± 0.09 $meq\cdot L^{-1}$ (mean \pm SE, $n = 5$) for solutions with pH 6.4 and 7.8, respectively.

Plants were irrigated twice daily for sufficient duration to completely saturate the sand. Solutions then drained to reservoirs below the tanks for reuse in the next irrigation. Calculations, accounting for maximum evapotranspiration, soil waterholding capacity, and intervals between irrigations, indicate that the salinity of the irrigation water (EC_w) was essentially equivalent to the salinity of the sand water (EC_{sw}). Based on a study of the soil-water dynamics in this river sand (Wang, 2002), EC_{sw} is ≈ 2.2 times the EC of the saturated soil extract (EC_e), the salinity parameter used to characterize salt tolerance in most studies (Ayers and Westcot, 1985). Our salinity treatments may be estimated as 0.91, 1.82, 2.73, 3.64, and 4.55 $dS\cdot m^{-1}$. Thus, the threshold EC_w at which marigold cultivars exhibited acceptable growth, 8 $dS\cdot m^{-1}$, would be equivalent to an EC_e of 3.64 $dS\cdot m^{-1}$ (Valdez-Aguilar et al., 2009b).

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Table 1. Composition of salinizing salts in solutions used to irrigate marigold grown in greenhouse sand tanks.

EC _w (dS·m ⁻¹)	Concn (meq·L ⁻¹)				
	Ca ²⁺	Mg ²⁺	Na ⁺	SO ₄ ²⁻	Cl ⁻
2	5.0	5.0	8.0	5.0	13.0
4	7.8	12.1	20.9	13.1	27.5
6	11.4	18.7	32.3	20.0	42.0
8	15.1	25.3	43.6	27.2	57.2
10	18.8	32.6	55.0	34.6	72.5

EC_w = electrical conductivity of water.

Plants were harvested when most of the flower heads were fully mature. Leaf samples from the main shoot (LMS) and from the lateral shoots (LLS) of 'Flagstaff' and 'Yellow Climax' were separated from the stems, whereas samples of 'French Vanilla' included both leaves from the main shoot plus from the lateral shoots. Leaves and stems were washed twice in deionized water, blotted dry, placed in paper bags, and oven-dried at 70 °C for 5 d. Once dried, the plant samples were ground to pass a 20-mesh screen. Total sulfur, total phosphorus, Ca²⁺, Mg²⁺, Na⁺, K⁺, Fe²⁺, Zn²⁺, Cu²⁺, and Mn²⁺ were analyzed on nitric-perchloric acid digests of the leaf and the stem tissues by inductively coupled plasma

optical emission spectrometry. Chloride was analyzed on nitric-acetic acid extracts by coulometric-ampereometric titration. The study was designed as a factorial experiment and set as a completely randomized design. Collected data and linear, quadratic, and cubic trends were analyzed by the analysis of variance procedure using SAS Version 8.2 (SAS Institute, Inc., 2001).

Results and Discussion

Calcium. Increasing EC_w and alkaline pH of irrigation water caused a general decrease in leaf Ca²⁺ concentration (Fig. 1). In the LLS of 'Flagstaff' and 'Yellow Climax', Ca²⁺ concentration was significantly affected by the pH of the irrigation water, but not by the pH × EC_w interaction, whereas in the LMS, there was a significant pH × EC_w interaction in both cultivars (Tables 2 and 3). The interaction in LMS was the result of the strong effect of pH resulting in higher Ca²⁺ concentrations when the substrate was acidic and EC_w was lower than 6 dS·m⁻¹. Increasing EC_w resulted in a significant decrease of Ca²⁺ in 'French Vanilla' (Table 4) and a significant interaction resulting from the lack of response

as EC_w increased up to 6 dS·m⁻¹ when pH was 6.4; Ca²⁺ decreased in a quadratic fashion in this cultivar when pH was 7.8 (Table 4; Fig. 1).

Main shoot leaves of 'Flagstaff' and 'Yellow Climax' accumulated higher Ca²⁺ at pH 6.4 compared with the LLS (Fig. 1). However, Ca²⁺ concentration was significantly reduced when EC_w rose to 8 or 10 dS·m⁻¹. The decrease in Ca²⁺ uptake may be the result of external ionic interactions with high salinity or the displacement of Ca²⁺ in the plasma membrane of root cells at high Na⁺ concentration (Cramer et al., 1985). Reduced plant growth of the three marigold cultivars was observed even at EC_w of 4 and 6 dS·m⁻¹, as reported in the companion paper (Valdez-Aguilar et al., 2009b); this response may be related to the osmotic effect of increasing salinity because a decrease in Ca²⁺ concentration was not detected at these EC_w levels. In addition, leaf dry weight (DW) production was significantly correlated with leaf Ca²⁺ content in all the three marigold cultivars (Table 5), suggesting that leaf Ca²⁺ accumulation was being regulated by growth.

Contrasting results concerning the effect of pH on Ca²⁺ accumulation have been

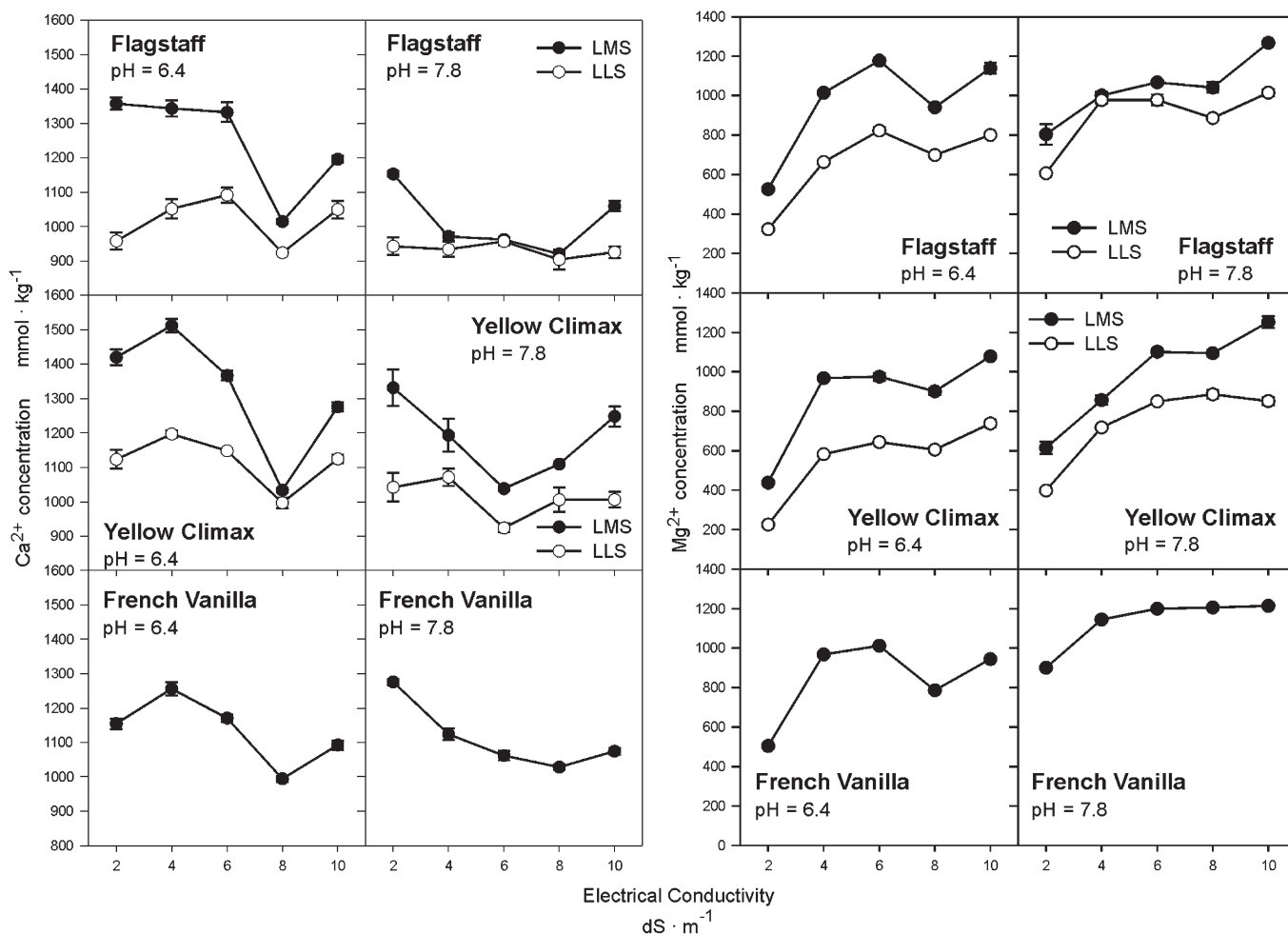


Fig. 1. Calcium and magnesium concentration in leaves of marigold *Tagetes patula* 'French Vanilla' or in leaves from the main shoot (LMS) and from the lateral shoots (LLS) in *Tagetes erecta* 'Flagstaff' and 'Yellow Climax' irrigated with water with increasing electrical conductivity and two pH levels. For some measurements, SE was smaller than the data point. Values are the means of three observations ± SE.

Table 2. Significance of trends and the effect of electrical conductivity of irrigation water (EC_w), pH, and $pH \times EC_w$ interaction on ion concentration in leaves from the main shoot (LMS) and lateral shoots (LLS) of marigold *Tagetes erecta* ‘Flagstaff’.

	Ca^{2+}		Mg^{2+}		Na^+		K^+		Total phosphorus		Total sulfur		Cl^-		Fe^{2+}		Cu^{2+}		Mn^{2+}		Zn^{2+}	
	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8
<i>LLS</i>																						
L^{zy}	NS	NS	***	***	NS	NS	***	NS	NS	NS	NS	*	***	***	NS	NS	*	NS	NS	NS	NS	NS
Q	NS	NS	***	***	NS	NS	***	*	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	***	NS
C	*	NS	***	***	NS	NS	***	NS	***	*	***	***	NS	NS	NS	NS	NS	NS	NS	NS	***	NS
EC_w	NS ²		***		NS		***		***		***		***		NS		***		NS		***	
pH	*		***		NS		NS		***		*		NS		NS		*		NS		***	
Int ^x	NS		***		NS		***		NS		*		***		NS		***		NS		***	
<i>LMS</i>																						
L^{zy}	***	**	***	***	NS	NS	***	NS	NS	NS	NS	***	***	***	NS	NS	*	*	NS	NS	NS	*
Q	NS	***	***	NS	NS	NS	**	NS	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
C	**	NS	***	NS	NS	NS	***	*	***	NS	*	NS	NS	NS	NS	NS	*	NS	NS	NS	***	NS
EC_w	**		***		NS		***		**		*		***		NS		*		NS		**	
pH	***		*		NS		NS		***		NS		NS		*		*		*		***	
Int ^x	***		***		NS		***		NS		NS		***		NS		NS		NS		***	

²L, Q, C = lineal, quadratic, and cubic trends, respectively.

^yNS, *, **, *** = Nonsignificant and significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

^xInteraction $pH \times EC_w$.

Table 3. Significance of trends and the effect of electrical conductivity of irrigation water (EC_w), pH, and $pH \times EC_w$ interaction on ion concentration in leaves from the main shoot (LMS) and lateral shoots (LLS) of marigold *Tagetes erecta* ‘Yellow Climax’.

	Ca^{2+}		Mg^{2+}		Na^+		K^+		Total phosphorus		Total sulfur		Cl^-		Fe^{2+}		Cu^{2+}		Mn^{2+}		Zn^{2+}	
	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8
<i>LLS</i>																						
L^{zy}	NS	NS	***	NS	NS	NS	***	NS	NS	NS	*	*	***	***	NS	NS	NS	NS	**	NS	NS	NS
Q	NS	NS	***	***	NS	NS	**	NS	NS	NS	NS	NS	NS	***	NS	NS	*	NS	NS	NS	*	NS
C	**	NS	***	***	NS	**	***	NS	*	NS	***	NS	NS	*	NS	NS	NS	*	NS	NS	*	NS
EC_w	NS		***		NS		***		*		**		***		NS		*		NS		NS	
pH	**		***		NS		NS		***		NS		**		NS		**		***		***	
Int ^x	NS		***		NS		**		NS		NS		***		NS		NS		*		NS	
<i>LMS</i>																						
L^{zy}	***	NS	***	***	NS	NS	***	NS	NS	*	***	**	***	***	NS	NS	NS	NS	**	NS	NS	NS
Q	NS	*	***	*	NS	*	***	*	*	NS	NS	NS	*	*	*	**	NS	NS	NS	NS	NS	NS
C	***	NS	***	NS	NS	NS	***	NS	**	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
EC_w	NS		***		NS		***		**		***		***		**		NS		*		NS	
pH	***		***		NS		***		***		NS		***		NS		NS		***		**	
Int ^x	***		***		NS		***		NS		**		***		NS		NS		*		NS	

²L, Q, C = lineal, quadratic, and cubic trends, respectively.

^yNS, *, **, *** = Nonsignificant and significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

^xInteraction $pH \times EC_w$.

Table 4. Significance of trends and the effect of electrical conductivity of irrigation water (EC_w), pH, and $pH \times EC_w$ interaction on ion concentration in leaves of marigold *Tagetes patula* ‘French Vanilla’.

	Ca^{2+}		Mg^{2+}		Na^+		K^+		Total phosphorus		Total sulfur		Cl^-		Fe^{2+}		Cu^{2+}		Mn^{2+}		Zn^{2+}	
	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8	6.4	7.8
L^{zy}	***	***	***	***	*	NS	**	*	NS	*	*	***	***	***	NS	NS	***	NS	NS	NS	NS	NS
Q	NS	***	***	***	NS	NS	*	NS	***	**	*	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
C	***	NS	***	*	*	NS	***	NS	***	NS	**	*	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
EC_w	***		***		NS		***		***		***		***		NS		NS		NS		**	
pH	NS		***		*		***		***		NS		**		NS		***		***		***	
Int ^x	***		***		*		***		***		***		NS		NS		**		NS		*	

²L, Q, C = lineal, quadratic, and cubic trends, respectively.

^yNS, *, **, *** = Nonsignificant and significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

^xInteraction $pH \times EC_w$.

reported. In the present experiment, Ca^{2+} concentration decreased only when EC_w was 8 $dS \cdot m^{-1}$ or higher, but in the LLS of ‘Flagstaff’ and ‘Yellow Climax’, Ca^{2+} concentration was unaffected compared with control plants (Fig. 1). When plants were irrigated with water of alkaline pH, Ca^{2+} concentration decreased when EC_w was 2 to 6 $dS \cdot m^{-1}$ in ‘Flagstaff’ and ‘Yellow Climax’ (or 4 to 6 $dS \cdot m^{-1}$ in ‘French Vanilla’); thus,

high pH stress induced a lower Ca^{2+} uptake even at lower salinity. This is in contrast to a report presented by Islam et al. (1980), which indicated increased Ca^{2+} in shoots of several species when pH was increased up to 8.5. Calcium acquisition may have been suppressed under high salinity as a result of interactions with other ions in the external solution, resulting in lower Ca^{2+} activity, but the combination of high salinity and high pH

may decrease Ca^{2+} activity at a greater extent as a result of the formation of calcium carbonates. When plants were exposed to the combination of alkaline pH and increasing EC_w , the concentration of Ca^{2+} in the younger leaves was not markedly different from that of plants irrigated with water at pH 6.4.

Magnesium. Magnesium concentration generally increased with increasing EC_w in all three marigold cultivars (Fig. 1; Tables 2–4). In

Table 5. Regression models for leaf content of Ca^{2+} , K^+ (mmol/plant), micronutrients (mg/plant), and leaf dry weight (Y), in grams, in three cultivars of marigold.^z

Nutrient content	Cultivar	Model	R ²
Calcium	Flagstaff	$Y = -0.331 + 1.031 \text{ Ca}^{2+}$	0.981
	Yellow Climax	$Y = -0.162 + 0.897 \text{ Ca}^{2+}$	0.983
	French Vanilla	$Y = 0.145 + 0.772 \text{ Ca}^{2+}$	0.963
Potassium	Flagstaff	$Y = 1.576 + 1.708 \text{ K}^+$	0.919
	Yellow Climax	$Y = 2.065 + 2.041 \text{ K}^+$	0.909
	French Vanilla	$Y = 0.624 + 1.270 \text{ K}^+$	0.821
Iron	Flagstaff	$Y = -0.325 + 5.017 \text{ Fe}$	0.871
	Yellow Climax	$Y = 0.360 + 7.757 \text{ Fe}$	0.931
	French Vanilla	$Y = 0.029 + 5.817 \text{ Fe}$	0.896
Zinc	Flagstaff	$Y = 0.879 + 66.240 \text{ Zn}$	0.972
	Yellow Climax	$Y = 1.706 + 45.981 \text{ Zn}$	0.944
	French Vanilla	$Y = 0.600 + 32.734 \text{ Zn}$	0.689
Copper	Flagstaff	$Y = -0.818 + 110.7 \text{ Cu}$	0.960
	Yellow Climax	$Y = 1.461 + 92.009 \text{ Cu}$	0.948
	French Vanilla	$Y = 0.423 + 79.767 \text{ Cu}$	0.891
Manganese	Flagstaff	$Y = 0.667 + 4.990 \text{ Mn}$	0.912
	Yellow Climax	$Y = 0.934 + 4.673 \text{ Mn}$	0.874
	French Vanilla	$Y = 0.562 + 2.382 \text{ Mn}$	0.691

^zIon content considered both leaves from the main stem and from the lateral stems (n = 60 for 'Flagstaff' and 'Yellow Climax'; n = 30 for 'French Vanilla').

'Flagstaff' and 'Yellow Climax', Mg^{2+} concentration was higher in the LMS than in LLS; however, the magnitude of the difference between leaf positions was larger when EC_w was higher than 4 $\text{dS}\cdot\text{m}^{-1}$. The combination of pH and EC_w treatments resulted in a significant interaction, which is explained by the different Mg^{2+} concentration resulting from the pH effect when plants were irrigated with nonsaline water (Fig. 1). In plants irrigated with acid water, a quadratic trend best described the increased Mg^{2+} concentration in response to higher EC_w . However, when pH was 7.8, the response was linear for the LMS and cubic for the LLS (Tables 2–4).

The increase in Mg^{2+} concentration was probably the result of external Mg^{2+} , increasing in concert with increasing EC_w (Table 1). It is well established that Mg^{2+} competes with Ca^{2+} (Marschner, 1995) by decreasing the membrane sorption capacity even at a higher extent than Na^+ (Yermiyahu et al., 1994). Because the activity of external Ca^{2+} may have been decreased at high EC_w , the competition at membrane level between Ca^{2+} and Mg^{2+} might have been lower, leading to an increase in the selectivity of Mg^{2+} over Ca^{2+} . Carvajal et al. (1999) reported that yield in hydroponically grown tomato was negatively correlated with increasing Mg^{2+} concentration in the external saline solutions, suggesting that the response was the result of nutrient imbalances because there was a clear $\text{Ca}^{2+}:\text{Mg}^{2+}$ interaction. In another study (Carvajal et al., 2000), this nutrient imbalance hypothesis was also supported because growth of tomato plants was partially restored under salinity treatments when the $\text{Ca}^{2+}:\text{K}^+:\text{Mg}^{2+}$ ratio of the external solution was more balanced (10:12:3) than when it was higher in Ca^{2+} and K^+ (4:6:1).

In general, alkaline pH of irrigation water was associated with an increase in Mg^{2+} concentration. The tendency to accumulate more leaf Mg^{2+} in plants irrigated with 2 $\text{dS}\cdot\text{m}^{-1}$ alkaline water, rather than under slightly acidic conditions, is in agreement with reports by Islam et al. (1980), who

observed this response in the shoots of ginger, cassava, tomato, French bean, wheat, and maize. Increased acquisition of Mg^{2+} may also be related to the decreased competition with Ca^{2+} resulting from the formation of calcium carbonates under high pH and/or a decrease in the efficiency of the H^+ pump at high H^+ concentration in the external medium (Marschner, 1995).

Potassium. In the LMS and LLS of 'Flagstaff' and 'Yellow Climax' as well as in the leaves of 'French Vanilla', concentration of K^+ decreased when plants were irrigated with EC_w 4 $\text{dS}\cdot\text{m}^{-1}$ or higher and acidic pH (Fig. 2; Tables 2–4). However, higher EC_w values did not result in additional reduction in K^+ concentration, explaining the cubic trends detected when pH was 6.4. The decrease in internal K^+ concentration has been associated with a competition between external K^+ and high external Na^+ in saline solutions (Grattan and Grieve, 1999). The interaction of alkaline pH and increasing EC_w was explained principally by the decrease in tissue K^+ concentration when EC_w was 2 $\text{dS}\cdot\text{m}^{-1}$ but pH increased from 6.4 to 7.8 (Fig. 2). In addition, in plants irrigated with acidic water pH, the effect of increasing EC_w on K^+ concentration was much higher than when irrigation water had alkaline pH.

Potassium tissue concentration exhibited a negative linear correlation with internal Mg^{2+} when plants were irrigated with pH 6.4 water. In 'French Vanilla', the correlation was significant ($r = -0.899^{***}$) as well as in the LLS of 'Flagstaff' ($r = -0.876^{***}$) and 'Yellow Climax' ($r = -0.921^{***}$) and in the LMS of 'Flagstaff' ($r = -0.901^{***}$) and 'Yellow Climax' ($r = -0.935^{***}$). However, when the solution pH was 7.8, the correlation was significant only for 'Yellow Climax' in the LLS and LMS ($r = -0.650^{**}$ and $r = -0.568^*$, respectively). The negative correlation suggests an antagonism between K^+ and Mg^{2+} as reported by Ding et al. (2006), Fageria (2001), Marschner (1995), and Troyanos et al. (2000). Results reported

by Grunes et al. (1992) and Huang and Grunes (1992) suggest that translocation of Mg^{2+} is negatively correlated with a high root K^+ concentration. Potassium content in LLS and LMS was positively correlated with leaf DW in all the three marigold cultivars (Table 5), indicating that the reduced growth may be an effect of impaired enzymatic activity or water relations in K^+ -deficient plants irrigated with high EC_w water.

Potassium concentrations in LMS were below the deficiency level for most of the cultivated plants. At harvest time, the LMS in 'Flagstaff' and 'Yellow Climax' developed a marginal chlorosis when plants were irrigated with acidic water and increased EC_w . According to Mills and Benton Jones (1991), a survey average K^+ concentration in mature *T. erecta* plants grown in 36-cell packs was reported to be 560 $\text{mmol}\cdot\text{kg}^{-1}$ (2.19%), whereas in *T. patula*, it was between 714 and 737 $\text{mmol}\cdot\text{kg}^{-1}$ (2.79% to 2.88%). In LMS of 'Flagstaff' and 'Yellow Climax', K^+ concentration was much lower than these levels when irrigated with high EC_w (Fig. 2); thus, the marginal chlorosis observed on these leaves may be the result of a deficiency of K^+ . Compared with the mature leaves of control plants under high pH, K^+ concentration was higher when plants were irrigated with 10 $\text{dS}\cdot\text{m}^{-1}$ water and yet, no deficiency symptoms were observed; this apparent contradiction may be the result of a concentration effect in K^+ attributable to the reduced leaf growth under high EC_w . The LLS did not exhibit symptoms of deficiency of K^+ , probably because K^+ was retranslocated from the mature leaves to supply the developing leaves. However, the overall effect on aesthetic value was negligible because the mature leaves were hidden by the leaves of the lateral stems.

Sodium. Leaf Na^+ concentrations were much lower than expected (Fig. 2) because high Na^+ concentrations in the nutrient solutions were used (Table 1). Increasing EC_w or pH of irrigation water in 'Flagstaff' and 'Yellow Climax' had a nonsignificant effect on Na^+ concentration (Tables 2 and 3); in 'French Vanilla', there was a significant but limited effect of water pH (Table 4). In general, Na^+ concentrations in marigold were very low as compared with reports for other ornamentals irrigated with water of high salinity. Sodium in leaves of *Limonium perezii* (Carter et al., 2005a) and *Celosia argentea* (Carter et al., 2005b), for example, increased from 700 to 1400 $\text{mmol}\cdot\text{kg}^{-1}$ and from 200 to 500 $\text{mmol}\cdot\text{kg}^{-1}$, respectively, in response to increasing Na^+ concentrations in saline irrigation waters. However, in our study, marigold concentration in foliar tissue ranged from 15 to 55 $\text{mmol}\cdot\text{kg}^{-1}$, suggesting that marigold possesses a mechanism that effectively restricts Na^+ transport to the leaves. Leaf Na^+ concentrations in the present experiment are in close agreement with the values reported by Jarecki et al. (2005) with levels of ≈ 44 to 130 $\text{mmol}\cdot\text{kg}^{-1}$ in shoots of marigold 'Crackerjack' irrigated with a half-strength Hoagland's solution containing

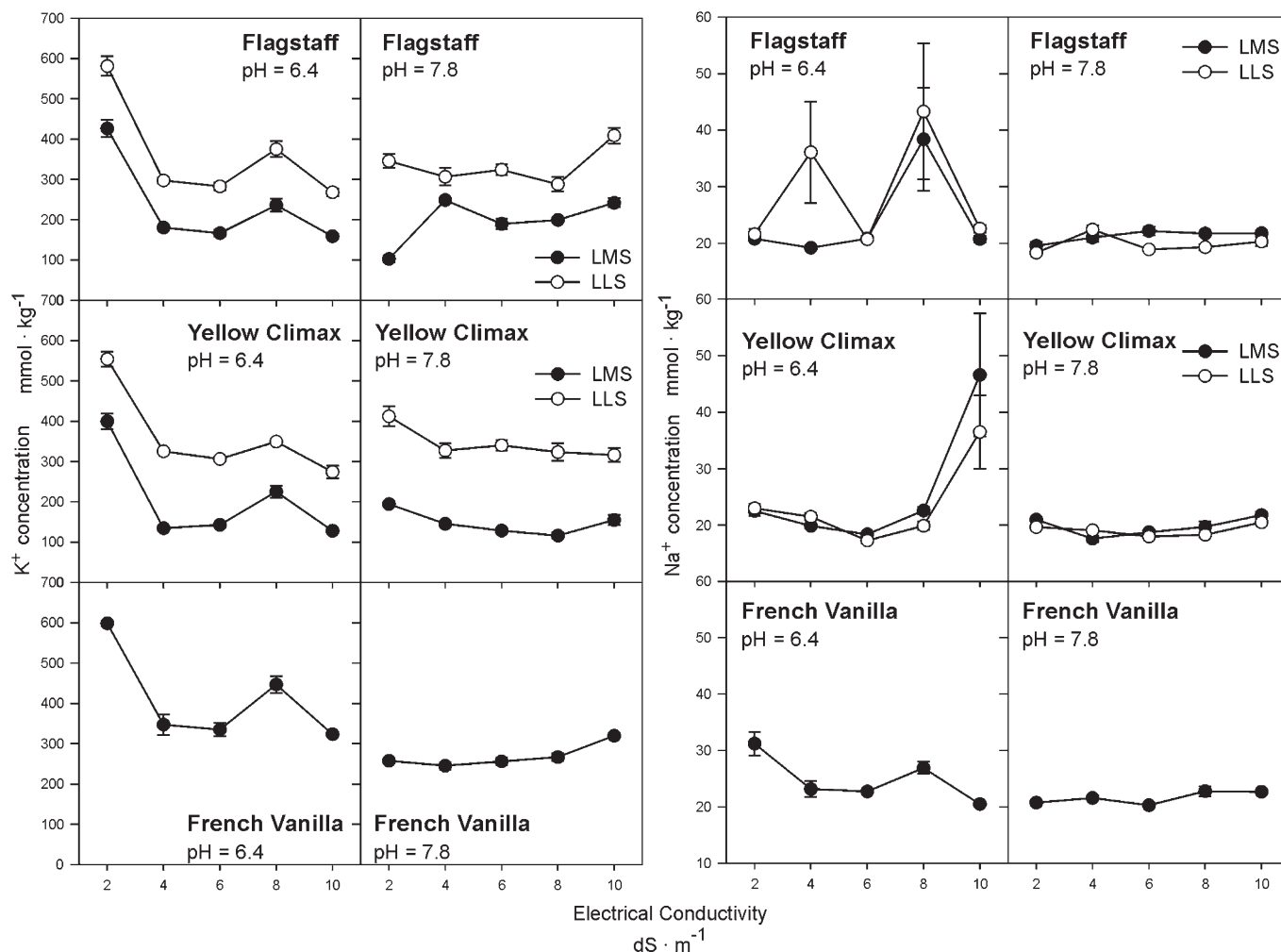


Fig. 2. Potassium and sodium concentration in the leaves of marigold *Tagetes patula* 'French Vanilla' or in the leaves from the main shoot (LMS) and from the lateral shoots (LLS) in *Tagetes erecta* 'Flagstaff' and 'Yellow Climax' irrigated with water with increasing electrical conductivity and two pH levels. Values are the means of three observations \pm SE.

8.4 meq Na^+/L . However, the authors reported root Na^+ concentrations of $\approx 740 \text{ mmol}\cdot\text{kg}^{-1}$, indicating that Na^+ was sequestered in the root. We also found evidence of limited partitioning of Na^+ stem tissues, which was effective until EC_w exceeded $6 \text{ dS}\cdot\text{m}^{-1}$ (Table 6).

Sodium exclusion capacity would explain the absence of correlation between leaf Na^+ concentration and plant growth (data not shown), although it is important to consider that the energy the plants must have spent for the exclusion of Na^+ may have negatively impacted growth. Our results are also in agreement with those reported by Huang and Cox (1988) who observed a slight (from 195 ppm in the control with EC_w $1.3 \text{ dS}\cdot\text{m}^{-1}$ to 215 ppm in plants irrigated with EC_w $7.9 \text{ dS}\cdot\text{m}^{-1}$ water) increase in leaf Na^+ concentration of marigold plants cultivated in a peat-based medium and irrigated with increasing concentrations of a $\text{NaCl} + \text{CaCl}_2$ mixture. Huang and Cox (1988) reported a 7.4 to 20.4 times higher Na^+ concentration in the roots than in the shoot of marigold, suggesting a restricted translocation of Na^+ under saline conditions.

High $\text{K}^+:\text{Na}^+$ ratios have been considered reliable markers for tolerance to salinity (Juan et al., 2005; Maathuis and Amtmann, 1999). Sodium exclusion in marigold may have increased the tolerance to salinity because K^+ was not significantly affected when EC_w increased from 4 to $10 \text{ dS}\cdot\text{m}^{-1}$ (Fig. 2); thus, a lower Na^+ concentration combined with a less affected K^+ may have resulted in a higher $\text{K}^+:\text{Na}^+$ ratio. In 'Flagstaff', the $\text{K}^+:\text{Na}^+$ ratio was 27.4 in the LLS of plants irrigated with water of EC_w $2 \text{ dS}\cdot\text{m}^{-1}$, but increasing salinity to $4 \text{ dS}\cdot\text{m}^{-1}$ caused a decrease to 11.8. Nonetheless, the $\text{K}^+:\text{Na}^+$ ratio was not further decreased as salinity of irrigation water rose to $10 \text{ dS}\cdot\text{m}^{-1}$ and varied between 11.8 and 14.9 (data not shown), a considerably higher value than that reported for *Matthiola incana* (Grieve et al., 2006).

Phosphorus and sulfur. EC and pH of irrigation water significantly affected total phosphorus (P) concentration in the three marigold cultivars (Tables 2–4), whereas the $\text{pH} \times \text{EC}_w$ interaction was significant only for 'French Vanilla' (Table 4). Leaf total P of plants irrigated with acid water increased in a cubic trend when EC_w

increased up to $6 \text{ dS}\cdot\text{m}^{-1}$, but when EC_w was higher than $8 \text{ dS}\cdot\text{m}^{-1}$, total P concentration decreased to the level of the control plants (Fig. 3). Total sulfur (S) exhibited similar responses as total P.

It has been proposed that P concentration increases in salinity studies performed in sand or solution cultures as a result of the higher P supply (Grattan and Grieve, 1999); however, our results contrast with other reports in which plants were grown under comparable conditions. Carter et al. (2005a, 2005b) reported a negative correlation between total P concentration and total S and Cl^- concentration when *Limonium perezii* and *Celosia argentea* plants were irrigated with water of similar composition to the one used in our study. Although Hu and Schmidhalter (2005) reported that competition between Cl^- and H_2PO_4^- is unlikely, several reports indicate that Cl^- reduces P (Papadopoulos and Rendig, 1983) and SO_4^{2-} uptake (Papadopoulos, 1987; Papadopoulos et al., 1985). Negative correlations between total P and total S and total P and Cl^- were also reported in *Ranunculus asiaticus* (Valdez-Aguilar et al., 2009a), suggesting some competition

between P and S or Cl^- . In contrast, in the present study, a positive relationship between total P and total S concentration was found in all the three marigold cultivars when irrigation water pH was 6.4 (Fig. 3), but not with Cl^- (Fig. 4). The correlation coefficients

between total P and total S concentration in 'Flagstaff' and 'Yellow Climax' were significant for the LMS ($r = +0.621^*$ and $r = +0.577^*$, respectively) and for the LLS ($r = +0.593^*$ and $r = +0.683^{**}$, respectively) and for 'French Vanilla' ($r = +0.661^{**}$).

Table 6. Significance of trends and the effect of electrical conductivity of irrigation water (EC_w), pH, and $\text{pH} \times \text{EC}_w$ interaction on sodium and chloride concentration in stems of three cultivars of marigold.

EC_w ($\text{dS} \cdot \text{m}^{-1}$)	Flagstaff				Yellow Climax				French Vanilla			
	Na^+		Cl^-		Na^+		Cl^-		Na^+		Cl^-	
	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
2	17.7	13.0	226	164	16.5	16.2	261	146	16.8	17.7	114	132
4	45.9	64.3	634	876	39.2	9.1	740	700	55.2	51.5	501	758
6	88.2	91.6	751	971	102.2	36.1	785	869	86.0	62.3	651	834
8	134.5	57.0	688	1035	179.0	28.1	801	1049	272.0	169.3	816	1116
10	180.3	282.7	767	1124	195.0	97.2	921	1225	236.0	272.3	887	1141
L_{xy}	***	***	***	***	***	***	***	***	***	***	***	***
Q	NS	*	***	***	NS	*	***	***	NS	NS	***	***
C	NS	*	***	***	NS	NS	***	**	*	NS	NS	*
EC_w	***	***	***	***	***	***	***	***	***	***	***	***
pH	NS	NS	***	***	***	***	***	***	NS	NS	***	***
Int^x	NS	NS	***	***	**	**	***	***	NS	NS	**	**

²L, Q, C = lineal, quadratic, and cubic trends, respectively.

³NS, *, **, *** = Nonsignificant and significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

⁴Interaction $\text{pH} \times \text{EC}_w$.

Increasing the pH of irrigation water to 7.8 significantly increased total P concentration in the three marigold cultivars (Tables 2–4) compared with plants irrigated with water of pH 6.4 (Fig. 3). The higher pH treatment was responsible for an increase in total P in the LLS of 'Flagstaff' and 'Yellow Climax' of 112% and 55%, respectively, whereas in the LMS, the increase was 223% and 128%, respectively. In 'French Vanilla', the increase in total P was 50%. Alkaline pH combined with increasing EC_w appears to invoke a synergistic effect because total P increased at a higher rate (Fig. 3). To a certain extent, this effect was unexpected because under alkaline conditions, the formation of calcium phosphates is higher, reducing availability of soluble P and reducing plant uptake and accumulation. However, total P accumulation [found by calculating total P content according to leaf dry weight (data not shown)] was not altered by pH, suggesting that pH of water increased total P concentration as salinity decreased plant growth. High alkalinity, however, decreased plant DW accumulation. Salinity also appears to affect total P concentration in the foliage through a decrease in plant growth. Total P in 'Yellow

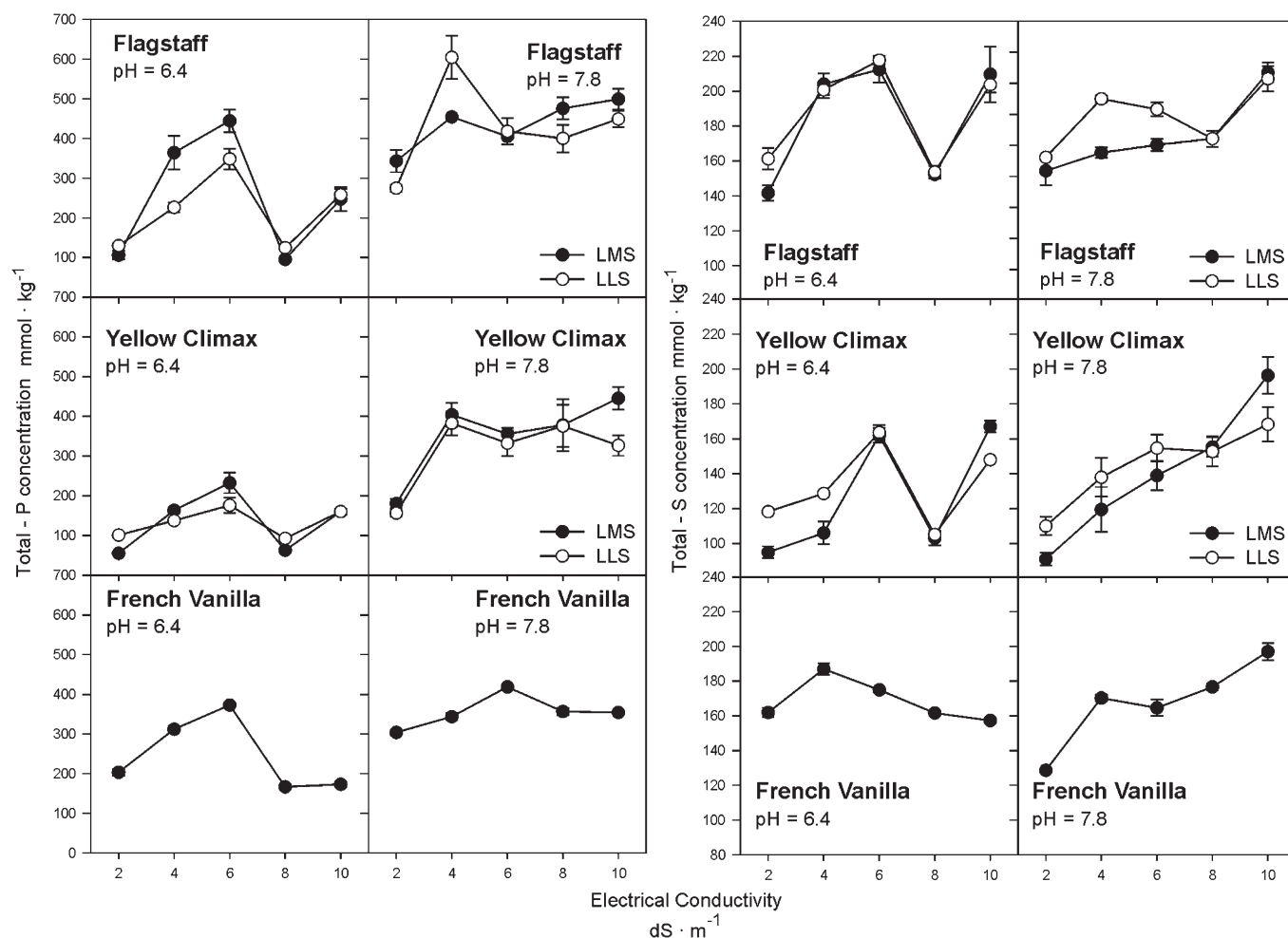


Fig. 3. Total phosphorus and total sulfur concentration in the leaves of marigold *Tagetes patula* 'French Vanilla' or in the leaves from the main shoot (LMS) and from the lateral shoots (LLS) in *Tagetes erecta* 'Flagstaff' and 'Yellow Climax' irrigated with water with increasing electrical conductivity and two pH levels. Values are the means of three observations \pm SE.

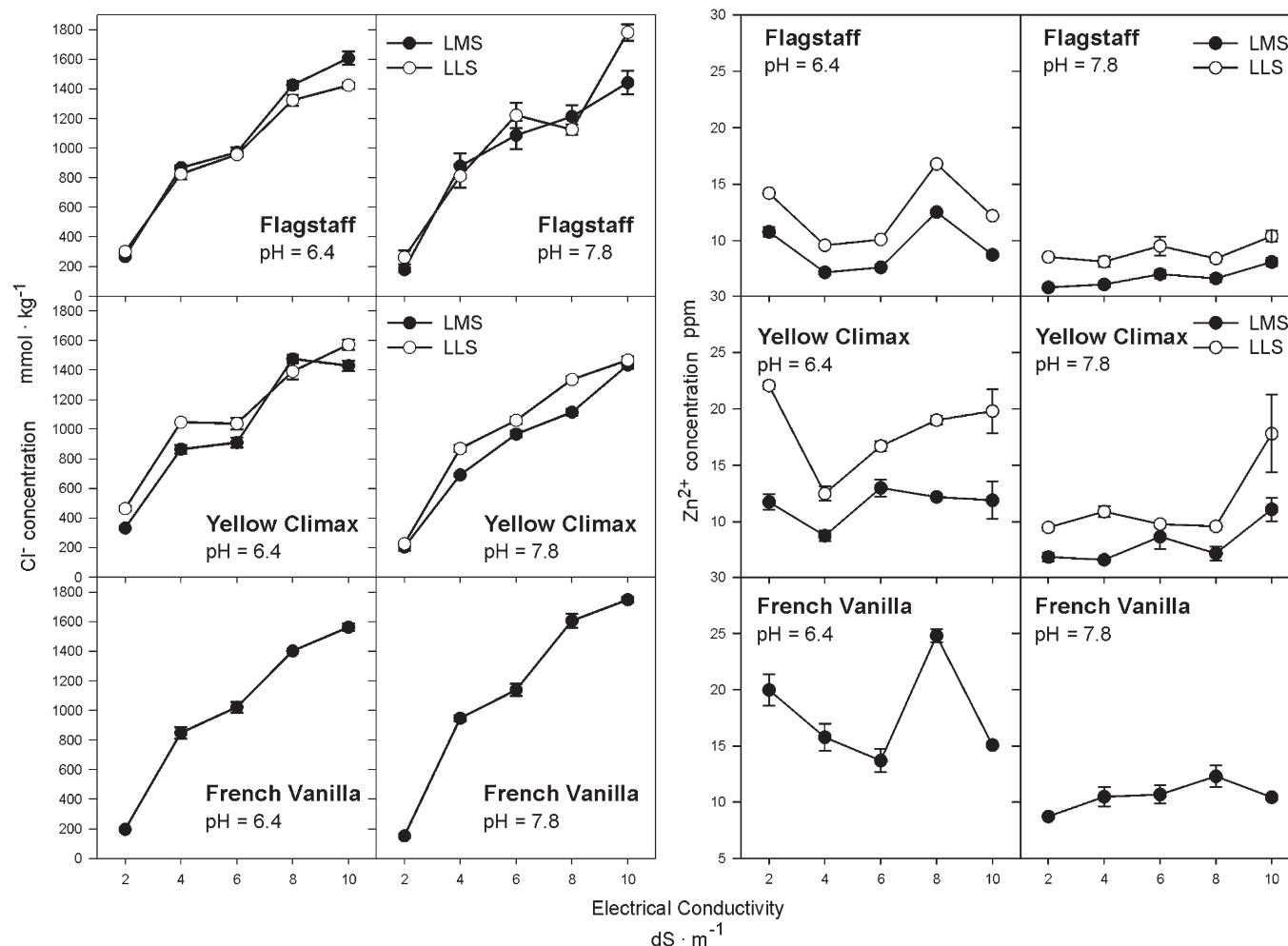


Fig. 4. Chloride and zinc concentration in the leaves of marigold *Tagetes patula* 'French Vanilla' or in the leaves from the main shoot (LMS) and from the lateral shoots (LLS) in *Tagetes erecta* 'Flagstaff' and 'Yellow Climax' irrigated with water with increasing electrical conductivity and two pH levels. Values are the means of three observations \pm SE.

Climax' varied between 0.97 and 1.60 mg/plant and 1.06 and 1.66 mg/plant when irrigation water pH was 6.4 and 7.8, respectively.

Chloride. Irrigation with saline water significantly increased Cl^- concentration in the three marigold cultivars (Tables 2–4; Fig. 4). The increase followed a linear trend and was significant when EC_w was 4 $\text{dS}\cdot\text{m}^{-1}$ or higher. The linear trend suggests that Cl^- uptake was a function of external concentration, as also reported by Hajrasuliha (1980) in bean plants. Leaf position had no effect on Cl^- concentration in individual leaves along the main shoot axis and indicates that the anion is highly mobile. Ben-Gal and Shani (2002) reported similar trends in tomato. Leaf and stem Cl^- concentrations increased as irrigation water salinity increased. In our study, internal Cl^- and Mg^{2+} concentration were correlated; in general, increasing Mg^{2+} was positively correlated with increasing Cl^- , which probably is a mechanism for the maintenance of charge balance. In 'Flagstaff' irrigated with water of pH 6.4, in the LLS, the correlation was $r = +0.817^{***}$, whereas in the LMS, it was $r = +0.730^{**}$. In the LLS of 'Yellow Climax', the correlation was $r =$

$+0.894^{***}$, whereas in the LMS, the correlation was $r = +0.773^{***}$. In 'French Vanilla', the correlation was $r = +0.653^{**}$. Similar correlations were observed when the pH of the irrigation water was 7.8.

Healthy leaves of pistachio trees contain 112 to 225 $\text{mmol}\cdot\text{kg}^{-1}$ Cl^- , whereas leaves containing 535 $\text{mmol}\cdot\text{kg}^{-1}$ exhibited severe marginal leaf scorching (Ashworth et al., 1985). Marigold exhibited a remarkable capacity to assimilate Cl^- , reaching concentrations close to 1500 $\text{mmol}\cdot\text{kg}^{-1}$. Because even the younger leaves contained high Cl^- levels, it is unlikely that Cl^- toxicity was responsible for the marginal chlorosis of the mature leaves mentioned previously. Salt-stressed tomato reportedly contained Cl^- concentrations in the range of 10 to 15 $\text{mmol}\cdot\text{kg}^{-1}$ in leaves, 8 to 10 $\text{mmol}\cdot\text{kg}^{-1}$ in stems, and 4 to 5 $\text{mmol}\cdot\text{kg}^{-1}$ in fruits (Ben-Gal and Shani, 2002). However, Pantalone et al. (1997) reported in susceptible soybean cultivars, leaf chlorosis was associated with Cl^- concentrations between 1507 and 2375 $\text{mmol}\cdot\text{kg}^{-1}$, whereas in tolerant cultivars, Cl^- ranged between 96 and 375 $\text{mmol}\cdot\text{kg}^{-1}$, probably as a result of an effective Cl^- exclusion mechanism. The Cl^- exclusion mechanism was not

detected in marigold as demonstrated by the concentrations in the stems (Table 6), which were comparable or even lower than those of the leaves.

Micronutrients. The effect of EC_w and pH on the internal Zn^{2+} concentration in marigold 'Flagstaff' (Table 2) and 'French Vanilla' (Table 4) was significant, whereas in 'Yellow Climax', Zn^{2+} accumulation was only affected by solution pH (Table 3). In general, the response of leaf Zn^{2+} to increasing EC_w was not consistent (Fig. 4); however, Zn^{2+} significantly decreased at a higher pH of irrigation water. Alkalinity is considered the major Zn^{2+} deficiency-causing factor in plants (Yang et al., 1993). Copper concentration was significantly affected by salinity of irrigation water in 'Flagstaff' and 'Yellow Climax' and by pH in all three marigold cultivars (Tables 2–4). As indicated for Zn^{2+} , Cu^{2+} concentration in response to increasing EC_w exhibited a nonconsistent pattern (Fig. 5); in general, the higher pH caused a significant decrease in Cu^{2+} content in LLS of 'Yellow Climax' and 'French Vanilla'. The pH effect was also associated with a decrease in internal Cu^{2+} concentration in the LLS of the three marigold cultivars. Internal

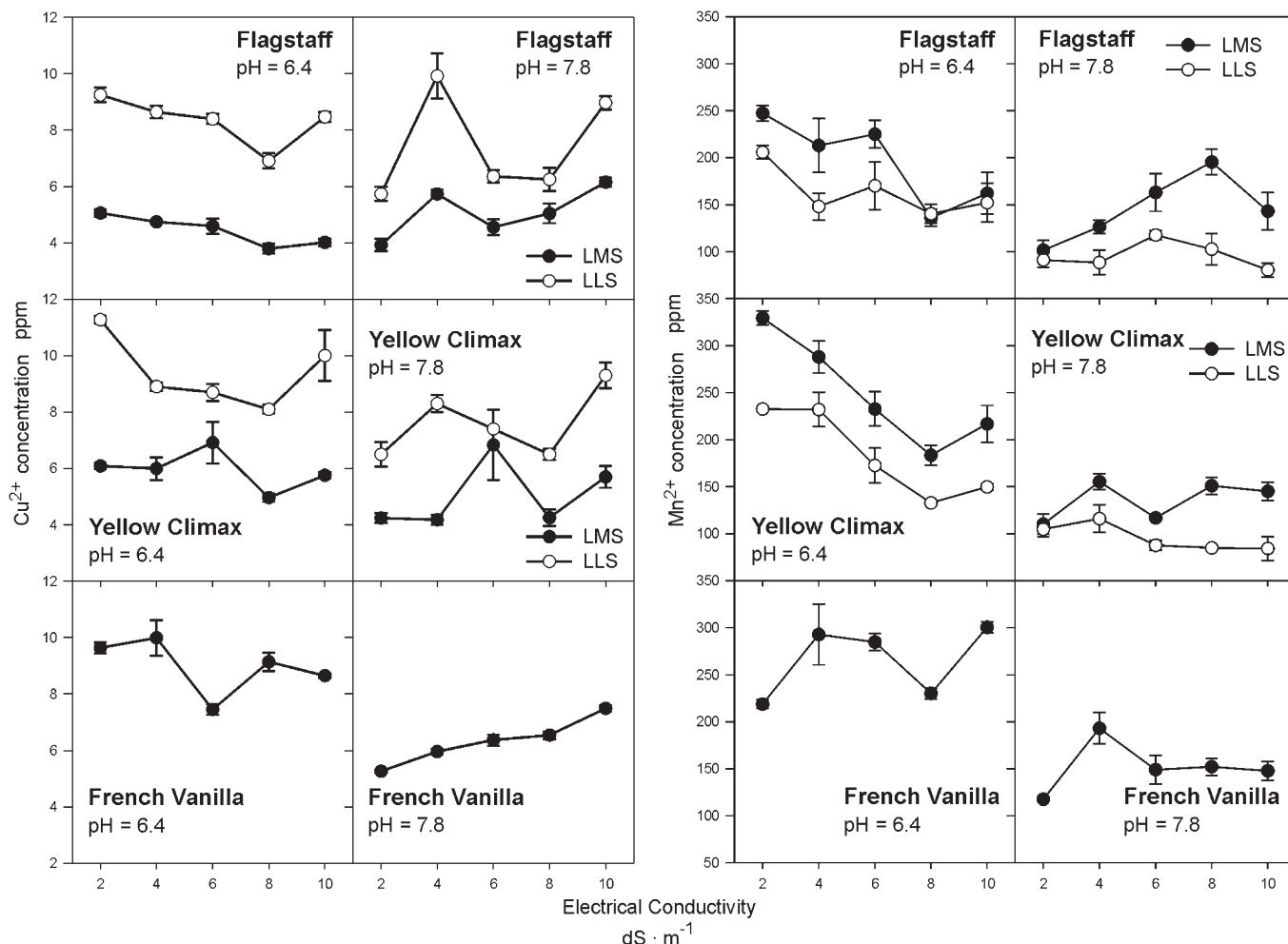


Fig. 5. Copper and manganese concentration in the leaves of marigold *Tagetes patula* 'French Vanilla' or in the leaves from the main shoot (LMS) and from the lateral shoots (LLS) in *Tagetes erecta* 'Flagstaff' and 'Yellow Climax' irrigated with water with increasing electrical conductivity and two pH levels. Values are the means of three observations \pm SE.

Mn^{2+} concentration tended to decrease with increasing EC_w in plants of 'Flagstaff' and 'Yellow Climax' when pH was 6.4, but this response was only significant in 'Yellow Climax' (Tables 2–4). As indicated for Cu^{2+} and Zn^{2+} , alkaline pH significantly reduced leaf Mn^{2+} concentration (Fig. 5). High irrigation water pH has been associated with decreased Fe^{2+} uptake and leaf chlorosis (Römheld, 2000). However, in the present study, internal Fe^{2+} concentration did not show a consistent trend with increasing salinity or pH of irrigation water (Fig. 6), perhaps as a result of the chelated Fe (Fe-DTPA) used in our nutrient solution.

In summary, increasing salinity appears to have little effect on micronutrient nutrition in marigold, a finding in agreement with reports by Eom et al. (2007) who found that NaCl salinity applied to six groundcover species had no effect on Fe^{2+} and Zn^{2+} accumulation, increased Mn^{2+} in *Alchemilla mollis* and *Solidago cutleri*, and decreased Cu^{2+} in *Nepeta x faassenii*. However, in our study, a higher pH in irrigation water caused decreased leaf Zn^{2+} , Cu^{2+} , and Mn^{2+} concentrations, which ranged between 50% to 223%, 22% to 445%,

and 46% to 67%, respectively. However, when micronutrient foliar content was calculated, a significant positive regression with leaf DW was detected (Table 5). No visual symptoms of micronutrient deficiencies were observed. Therefore, the positive correlation may be related to the lower growth rate resulting from the osmotic effects of salinity.

Plant growth, aesthetic value, and nutrient status. The effect of increasing EC_w and pH on plant shoot DW is reported in a companion paper (Valdez-Aguilar et al., 2009b). Internal K^+ concentration and shoot DW production were positively correlated (Fig. 6), suggesting that the reduction in K^+ resulting from higher salinity was associated with lower DW. On the other hand, a negative correlation between Mg^{2+} and shoot DW (Fig. 6) indicates that the increase in internal Mg^{2+} was associated with growth reduction. Similarly, shoot DW exhibited a quadratic decrease with internal Cl^- concentration (Fig. 6), corroborating that marigold, in common with other crops, is very sensitive to high Cl^- (Grattan and Grieve, 1999). Estañ et al. (2005) reported a negative correlation be-

tween leaf Cl^- concentration and yield of tomato plants grown at high NaCl concentrations, indicating that the most productive plants were those with the ability to regulate the transport of toxic ions. These correlations were not detected in marigold plants irrigated with water with pH 7.8. Martin and Koeberner (1995) indicate that in bean plants grown under high salinity, Cl^- exerted a higher toxicity than Na^+ , reducing plant growth; this was explained by the increased influx of Cl^- resulting from the perturbation of Ca^{2+} homeostasis in the plasmalemma. The authors also reported that Mg^{2+} was phytotoxic under such conditions.

Although plant growth was significantly reduced as salinity increased in waters with pH 6.4, the aesthetic quality of these marigold cultivars was not compromised, making them valuable for landscape sites where degraded waters are used for irrigation. The overall health of the marigold species was undoubtedly the result of favorable ion balances and interactions within the plant, including the nonsignificant effect on Ca^{2+} in the LLS of 'Flagstaff' and 'Yellow Climax' (Fig. 1), the attenuation of Mg^{2+}

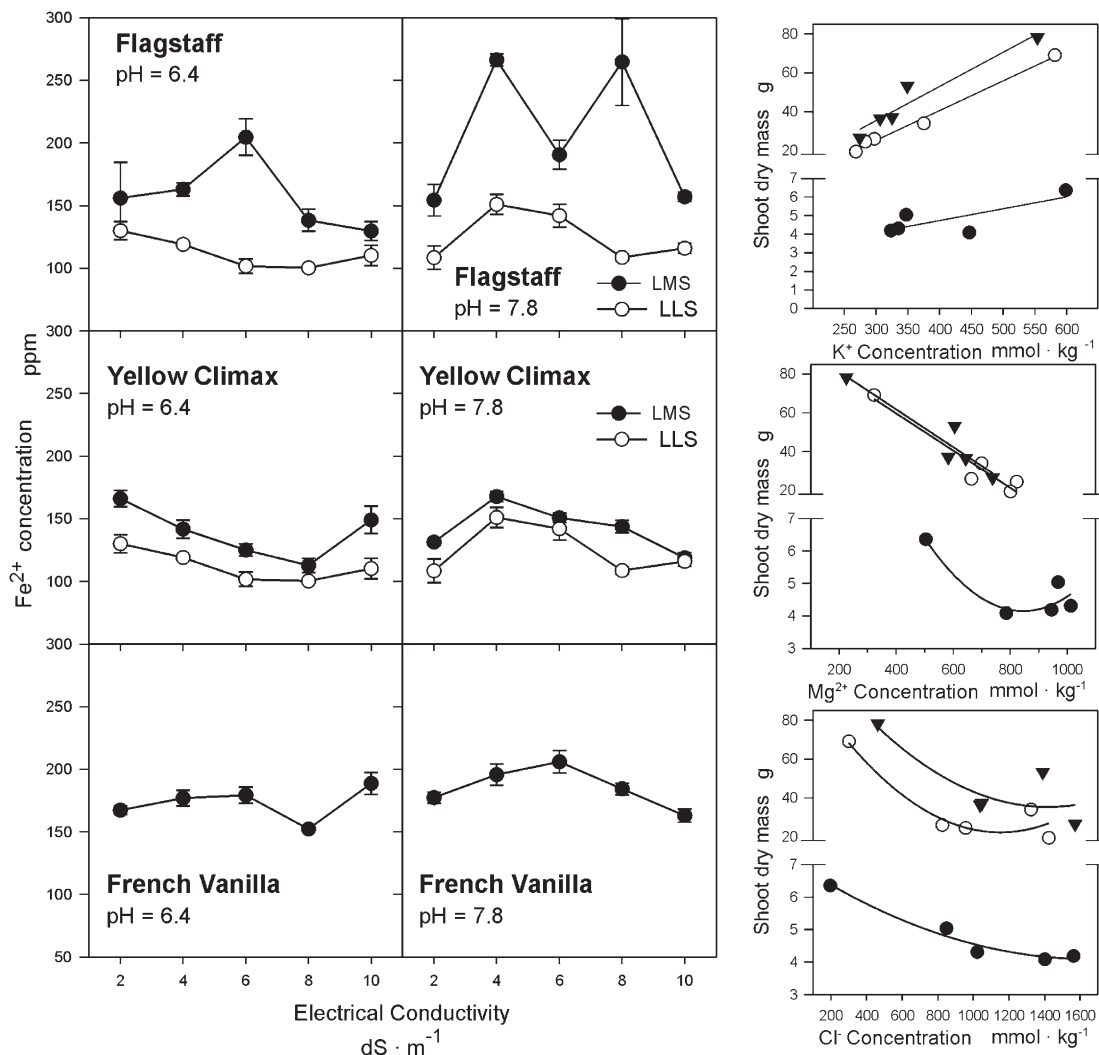


Fig. 6. Iron concentration in the leaves of marigold *Tagetes patula* 'French Vanilla' or in the leaves from the main shoot (LMS) and from the lateral shoots (LLS) in *Tagetes erecta* 'Flagstaff' and 'Yellow Climax' irrigated with water with increasing electrical conductivity and two pH levels. Values are the means of three observations \pm se and relationship between K^+ , Mg^{2+} , and Cl^- concentration in the leaves of marigold 'French Vanilla' (●) or the leaves from the lateral shoots of 'Flagstaff' (○) and 'Yellow Climax' (▼) and the shoot dry weight produced at experiment termination.

accumulation (Fig. 1) or decrease in K^+ (Fig. 2) when EC_w was 4 $dS\cdot m^{-1}$ or higher, and the effects on total P concentration in response to increasing EC_w (Fig. 3), and Na^+ exclusion, which resulted in a low leaf $Na^+:K^+$ ratio and a high selectivity for K^+ .

Conclusions

Growth of marigold plants irrigated with water at pH 6.4 significantly decreased as EC_w increased; however, the aesthetic value of the plants was not detrimentally affected. As suggested in a companion paper (Valdez-Aguilar et al., 2009b), growth reduction may be associated with the osmotic effects when plants were irrigated with solution of high EC_w . The maintenance of a high ornamental value in flowering stems of the salt-stressed marigolds may have been associated with specific mineral ion relations such as the nonsignificant effect on Ca^{2+} in the LLS of 'Flagstaff' and 'Yellow Climax', the attenuation of Mg^{2+} accumulation, the plateau in K^+

reduction when EC_w was 4 $dS\cdot m^{-1}$ or higher, and the exclusion of Na^+ . Considering only the pH effect, it caused a severe reduction in landscape attributes of marigold, which was associated with a significant decrease in K^+ , Zn^{2+} , and Cu^{2+} concentration and a significant increase in Mg^{2+} .

Aesthetic factors for evaluating ornamental landscape plants include attractiveness, size, and health. In some cases, growth may be reduced as much as 50% as long as plant appearance remains acceptable. Maximum permissible salinity levels for plants grown in landscape sites are generally based on soil salinity [EC of the saturated soil paste (EC_e)]. As a result of the soil–water dynamics of our sand tank system, the EC_w of the irrigation waters is ≈ 2.2 times that of the EC_e . The marigold cultivars retained aesthetic value and exhibited acceptable growth when EC_w was as high as 8 $dS\cdot m^{-1}$ (equivalent $EC_e = 3.64 dS\cdot m^{-1}$). Although many environmental and edaphic factors will influence the response of the marigold to salinity, the

cultivars are recommended for moderately saline landscape settings.

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